In-situ Chemical Imager[1]

Dmitri A. Kossakovski
Jet Propulsion Laboratory
California Institute of Technology
MS 306-336, 4800 Oak Grove Dr.
Pasadena, CA 91109
818-354-9575
dmitri.kossakovski@jpl.nasa.gov

Gregory H. Bearman
Jet Propulsion Laboratory
California Institute of Technology
MS 306-336, 4800 Oak Grove Dr.
Pasadena, CA 91109
818-354-3285
gregory.h.bearman@jpl.nasa.gov

Joseph L. Kirschvink
Division of Geology and Planetary Sciences
California Institute of Technology
MS 170-25 Pasadena, CA 91125
626-354-6136
kirschvink@caltech.edu

Abstract—A variety of *in-situ* planetary exploration tasks such as particulate analysis or life detection require a tool with a capability for combined imaging and chemical analysis with sub-micron spatial resolution.

In our instrument, spatially resolved chemical imaging is achieved by augmenting a fiber optic scanning probe microscope with laser induced breakdown spectroscopy. Elemental composition of surfaces can be mapped and correlated with topographical data.

The study is conducted in ambient conditions or under vacuum with minimal sample preparation. A sharp fiber optic probe is used as a means for topographical analysis and as a delivery tool for pulsed laser radiation which vaporizes material below the probe. Optical emission from the ablation plume is analyzed with a UV/VIS spectrometer equipped with an intensified CCD detector. The ablation crater size is controlled by the amount of laser power coupled into the probe. Sampling areas with sub-micron dimensions are achieved by using reduced laser power.

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1. INTRODUCTION

What are the signatures of fossil life and how do we detect them? Our knowledge of these is predicated upon experience with terrestrial life as well as some basic chemistry that is expected to apply everywhere. For example, terrestrial life widely uses phosphate groups for energy [ATP], since the superphosphate bond can store a convenient amount of chemical energy. Extraterrestrial life may use other approaches, but the chemistry is universal and phosphorus is a likely choice. Similarly, other important elements for biological organisms are carbon, nitrogen, and sulfur. Figure 1 shows the elemental distribution in the earth's crust and terrestrial life. It is the *differential* elemental composition that is most useful as a biotic signature. Comparison of the composition of an object with its background matrix provides a clue of its origin. Trace element enrichment by biological processes can be in excess of 100 ppm [1].

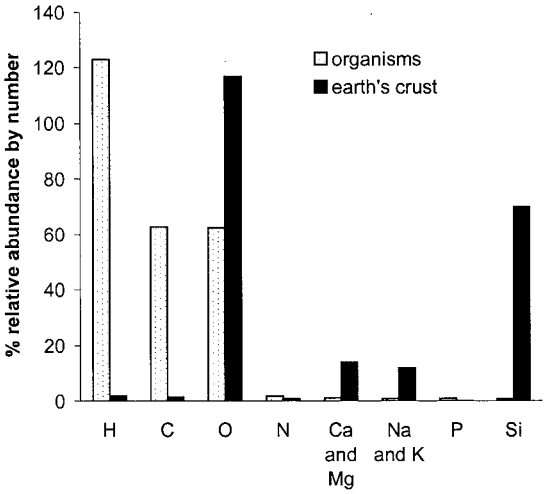

Figure 1. Relative abundance of elements in biological organisms and the crust of the earth. Differences in elemental abundance between an object and its background matrix are possible indicators of a biotic origin.

A third fossil biotic signature is a fossilized structure or the remains of biomineralization. One looks for the fossil remnants of organisms and bases that identification on the morphology of the remains. Earth provides many examples of such microfossils [2] in which morphology can be tied to an organic origin. However, it is not always clear that a microstructure pattern or regularity can be tied to a biogenic

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origin. It is possible for geochemical processes to effectively mimic such structure and produce analogous objects that are very difficult to differentiate from biotic ones [3,4]. In fact, there is a class of imposters known as "dubiofossils" to fool the unwary [5]! In 1996 Grotzinger and Rothman [6] demonstrated that an abiotic growth process could reproduce the morphology of fossil stromatolites that "combines chemical precipitation of the growing interface, fallout and diffusive rearrangement of suspended sediment".

How do we use these potential signatures as part of an organized search for extraterrestrial life? We must use multiple criteria for a convincing demonstration of a biogenic origin for an object. Since imaging of morphology alone can lead to identification errors, we propose to combine imaging with measurements of chemical composition. In turn, chemical composition alone without an imaging context is also only part of the picture. Knowledge of elemental enhancement for a sampled spot is misleading since imaging may show that one is sampling a thermally processed inclusion. The combination of both spatial and chemical information provides complementary information that yields a much more complete picture than either technique does alone.

We are not claiming that this instrument system will be able to unambiguously detect any and all biomineral products produced by living organisms. Indeed, some Earthly biomineral products are produced with little to no biological control on their formation processes, with the minerals merely being induced to grow as byproduct of the biological activity of the nearby organism. These 'biologically induced' products include the prodigious quantities of extracellular magnetite and pyrrhotite generated by dissimilatory iron reducing bacteria, as well as most Mn-precipitating bacteria. Unless observed directly forming as a result of biological activity, these will be hard to identify uniquely, either on Earth or Mars.

On the other hand, many biomineral products are formed internally under strict genetic and biological control, with the biomineral constituents often assuming morphologies and chemical compositions radically different from those of their biologically-induced or inorganic counterparts. Examples of this include virtually all of the carbonate and phosphate minerals produced by higher organisms (e.g., numerous examples in the book by Lowenstam and Weiner [7]) as well as the magnetite and greigite in the magnetosomes of the magnetotactic bacteria.

As a further example of how chemical imaging helps provides multiple criteria for biogenic activity, consider terrestrial magnetotactic bacteria. Magnetite produced by such bacteria is very pure chemically compared to magnetite produced through weathering and other geological processes. It does not contain typical trace elements such as Ti, Cr, Mn and Al [8,9,10]; these are apparently excluded by the biological membrane that surrounds the growing magnetite crystal, although they are available in solution [11]. These impurities reduce the saturation magnetization of magnetite and reduce its utility to the bacteria and are consequently selected against. The presence of these trace elements in an iron oxide sample rules it out as a possible magnetosome.

Our approach to spatially resolved chemical imaging is to combine scanning probe microscopy with chemically sensitive techniques. Technologies already exist to obtain either information set individually but not together. For example, AFM and other scanning probe microscopies will provide topographical information but nothing on chemical composition.

Conversely, laser desorption or ablation methods can provide chemical data but at the expense of imaging and topography for provide context. For example, when pulsed laser radiation is delivered to a sample it will ablate and atomize material in a plasma plume. Spectroscopic analysis of the plasma is generally referred to as Laser Induced Breakdown Spectroscopy, LIBS [12]. Emissions from the pulsed plasma can be analyzed to discern the chemical composition of the surface.

We are developing an instrument which combines LIBS with a scanning probe microscope to perform topographical and chemical imaging of surfaces and particles. In the our Chemical Imager a sharp fiber optic probe coupled to a pulsed laser is scanned across the sample to study topography. The probe is then positioned above features of interest in the topographical image and LIBS data recorded to provide chemical information to further characterize the sample. When specific chemical signatures are observed they can be monitored during a rescan of the surface to generate an image of the identified species.

2. INSTRUMENT DESIGN

The instrument is based on the concept reported previously [13]. Instrument diagram is shown in Figure 2.

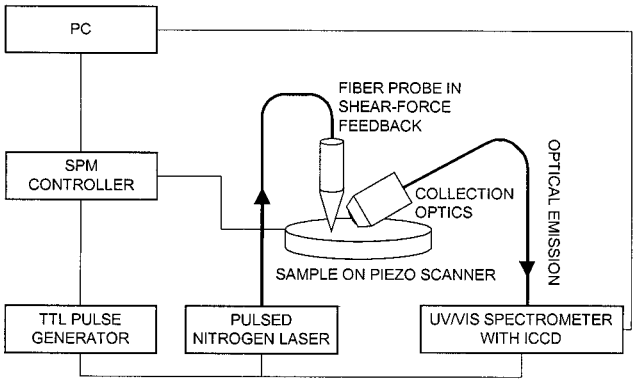

Figure 2. Schematic diagram showing major components of the Chemical Imager.

Scanning Probe Microscope

Our scanning probe microscope uses uncoated heat-pulled fiber optic probes and tuning fork position feedback. The fibers were pulled with a modified Sutter micropipette puller made by Sutter Instruments (Novato, CA). We have utilized multimode UV transparent fiber HFS-50 from Radiant Communications (South Plainfield, NJ), with fiber core and cladding diameters of 50 and 125 μm, respectively. A photograph of a pulled probe is shown in Figure 3a.

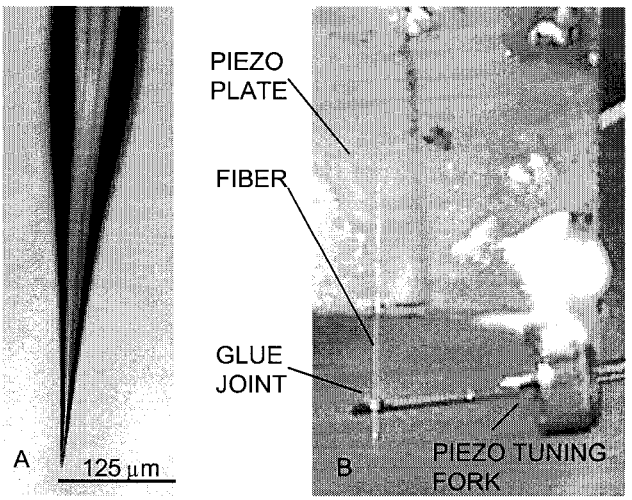

Figure 3. (a) Picture of a pulled fiber optic probe. The starting cladding diameter is 125 μm. The radius of curvature at the end of the taper is less than 1 micrometer. Most of the light efficiently propagates to the very tip of the taper. (b) Optical fiber glued to a high quality piezo resonator, piezo tuning fork, which in turn is attached to a larger piezoelectric plate. The plate is driven at the resonance frequency of the tuning fork.

Shear force feedback was implemented using piezoelectric tuning forks as a sensor element following Karrai [14]. In this methodology the probe is attached to the tuning fork which is a high quality resonator. Mechanical vibration of the probe at the resonance frequency of the fork is excited by a separate piezo element. The voltage output of the piezo fork is proportional to the amplitude of probe vibration. When the probe approaches the sample to the distance smaller than ~40 nm, this vibration is damped by probe-sample interaction. The amplitude of the electrical signal from the fork is also reduced. This signal is used for the distance feedback loop control.

Tuning forks with fundamental frequency of 32.768 kHz from Epson Electronics America (El Segundo, CA), part C-001R 32.768K-A, were used for the experiment. After the plastic coating was removed, fibers were heated and pulled so that a sharp taper is created, and then glued to the fork by cyanoacrylate. An assembled tip/probe system is shown in Figure 3b.

The operation of the scanner is controlled by a PScan2 SPM controller from Pacific Scanning (Pasadena, CA). The signal access console of the PScan2 controller is used to feed the output of the tuning fork circuit into the controller and also to provide master TTL pulses for the LIBS system.

The sample was mounted horizontally on a modified PTrak3 scanner from Pacific Scanning. The range of scanning is in excess of 90×90 μm in lateral directions (designated XY) and 12 μm in vertical direction (designated Z). Inductive sensors are used for scanner linearization along X and Y axis. This is essential for reliable positioning of the probe for chemical study of specific features. The scanner was suspended on bungee cords to reduce mechanical vibrations.

Laser Induced Breakdown Spectrometer

A pulsed nitrogen laser from Laser Science Inc. (Franklin, MA), model VSL-337, was used to perform LIBS experiments. The laser emits at 337 nm and delivers laser pulses of 120 μJ with duration of 3 ns, at a repetition rate of 20 Hz. The non-Gaussian beam is rectangular shaped with ~1×8 mm beam cross-section.

The beam was coupled into the fiber with a coupler from OZ Optics (Canada). Transmission efficiency of the uncoated tapered fiber was 5% or less, measured in far field.

It is difficult to measure surface power density of laser radiation delivered to ablation spot through a fiber probe since not all light emitted from the probe contributes to the plume formation. Our experiments employ uncoated probes, from which a fraction of the light escapes through the tapered sidewalls near the probe tip. Without knowing the percentage of the light lost through sidewalls, we can only estimate an upper limit of the surface power density in the TOPOLIBS experiment. Assuming 5% transmission through the fiber and 3 μm spot size, the surface power density is about 1.25×10^9 W/cm^2 which is above a typical threshold value for LIBS experiments [**Error! Bookmark**

not defined.]. This range of values is consistent with power densities required for observation of LIBS spectra. The issue of the spot size is considered in more detail in the Discussion section below.

Optical emission from the plasma plume was collected with a standard 20 power microscope objective, coupled into a 400 μm fiber and delivered to the 25 μm entrance slit of an MS125 spectrograph from Oriel (Stratford, CT).

The spectrograph was equipped with an intensified CCD (ICCD) detector from Andor Technology (Belfast, Northern Ireland). The ICCD can be triggered and gated with 25 ns resolution. This feature is very important for acquisition of good quality LIBS spectra because the plume emission has short lifetime and should be separated from the original laser pulse.

The timing scheme for the experiment is as follows. A TTL pulse from the PScan2 controller is delivered to digital delay generator DIG535 from Stanford Research Systems (Sunnyvale, CA). The delay generator supplies separate TTL pulses to the laser and ICCD, the delay between these pulses is adjustable.

Spectra were analyzed with GRAMS/32 package by Galactic Industries (Salem, NH). Tables of Spectral Line Intensities by W.F.Megger [15] and FindEM software package from Multichannel (Stockholm, Sweden) were used to assign spectral features.

3. Preliminary Data

Topography: Tool for Finding Interesting Features

In order to gain experience with diverse samples potentially interesting for *in-situ* analysis, we have collected a number of scientifically relevant samples, including fossils, planetary material analogs and meteorites.

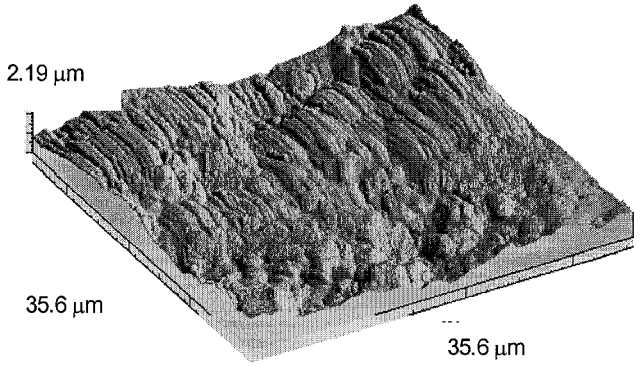

Figure 4. Topography of the *Bacculites rex* aragonitic shell. The shell is iridescent in visible light. Quazi-periodic deposits on the shell surface are responsible for this diffraction effect. The ordered deposits are well resolved in this topographic scan obtained with a sharp fiber optic probe.

For example, Figure 4 shows micro topography of an 80 Million year old aragonitic shell, *Bacculites rex.* The shell is iridescent in visible light which is explained by quazi-periodic deposits on the shell. These deposits act as a diffracton grating. Topographical scan with a pulled fiber probe clearly shows these ordered structures on the shell surface. Getting such data from an unknown sample would prompt further investigation with a need for chemical analysis of different regions, both structured and unstructured.

Spatial resolution of topographical imaging is determined by the sharpness of the probe. Typically, our probes have an end radius of curvature of about 100 nm when fresh. The probes however may be damaged in the course of the experiment effectively lowering spatial resolution.

LIBS: Tool for Chemical Analysis

Chemical analysis of the sample is performed after topographical study. The probe is positioned in the region of interest and laser pulse is fired. Figure 5 shows LIBS spectrum generated by a single laser pulse delivered to the *Bacculites rex* shell surface through the same fiber optic probe with which the topography analysis was performed. The probe was about 20 nm above the sample.

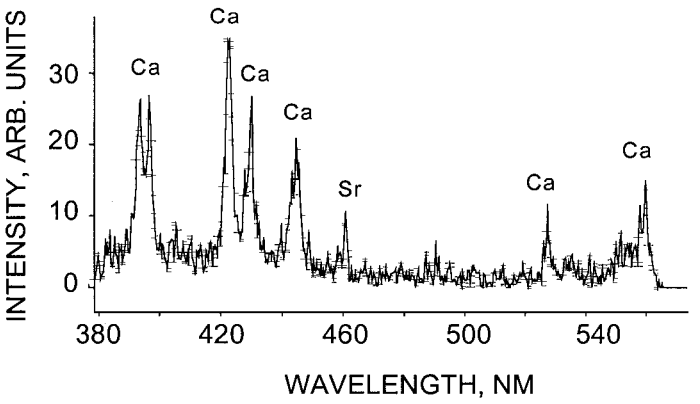

Figure 5. LIBS spectrum generated by a single laser pulse delivered to the *Bacculites rex* shell surface through the same fiber optic probe with which the topography analysis was performed. The probe was about 20 nm above the sample.

Multiple strong calcium lines are identified in the spectrum. Enhanced levels of Sr are commonly present in unaltered Ca-biominerals produced by marine organisms [**Error! Bookmark not defined.**].

At the time of the writing we do not have a capability for localized chemical analysis correlated with topographical map, which is the ultimate goal for Chemical Imager. This capability will be implemented shortly.

On The Way to Chemical Imaging

Among the issues that are critical for obtaining useful data with the Chemical Imager are:

- High resolution LIBS spectra: every emitted photon is valuable since single laser pulses are used to produce plasma.
- High sensitivity and fast detectors: majority of biologically important elements are weak LIBS emitters.
- High efficiency collection optics for LIBS signal acquisition: once again each photon is precious, especially if small laser powers are used to minimize sample damage. Reduced laser power leads to reduced amount of LIBS signal.
- Probe reliability and robustness: single probe should be able to serve long time for high quality scanning and laser radiation delivery. Mechanisms for probe exchange should be developed for remote/autonomous operation.

Sections below address the issues of spectrum signal-to-noise ratio and sample damage by laser pulses.

Time Resolved LIBS Spectra—In the initial stages of plasma formation the peaks from elemental emissions are masked by broadband bremsstrahlung radiation. Later in the plasma plume lifetime the background dies out and sharp spectral peaks from excited neutral species start dominating the spectra. This is the reason for having a fast, gateable detector such as ICCD. Figure 6 shows two spectra from the same sample, one acquired without time delay between plasma generation and spectrum acquisition, while another with 40 ns delay.

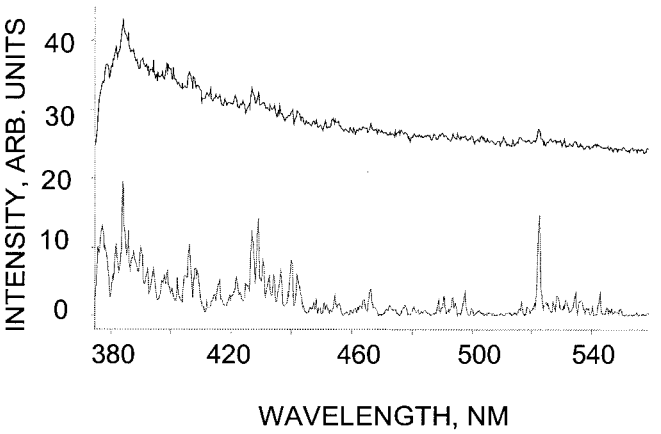

Figure 6. Comparison of LIBS spectra acquired with and without delay after the laser pulse. No delay for top spectrum translates into high background and low signal to noise ratio, SNR. Bottom spectrum was acquired with a delay of 40 ns after the laser pulse. SNR is dramatically better. Polished aluminum is a sample in both cases. Top spectrum is raised for presentation purposes.

The top spectrum was acquired by triggering the spectrometer before laser pulse, while the bottom one was acquired with a 40 ns delay after the laser pulse. The major emission lines are present in both spectra but the background signal is much lower in the "delayed" spectrum.

Crater sizes—LIBS is a destructive technique on a micro-scale. The size of a crater formed when laser pulse evaporates material depends on a number of factors such as:

- laser pulse characteristics - power and wavelength;
- size of the area on the sample where surface power density exceeds threshold value;
- material properties.

Figures 7 and 8 show different size of craters created by similar power laser pulse delivered to the surfaces of basalt and aluminum, respectively. Very clean, compact crater is created on basalt, while in case of aluminum the crater is much larger due to low melting point of the material.

These data indicate that in an experiment with unknown substance the laser power should be kept initially low and then gradually increased in order to produce detectable spectra with minimal sample damage.

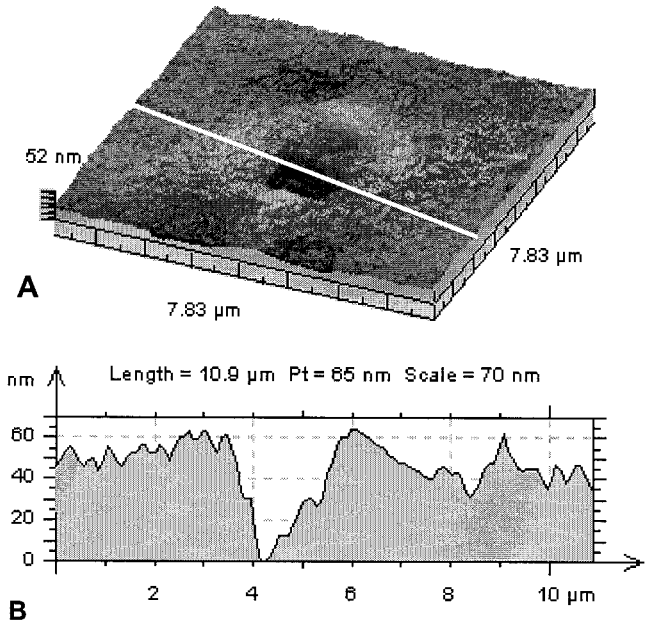

Figure 7. (a) Topography of a small crater created by a single laser pulse delivered through fiber probe to the surface of polished basalt. (b) Cross section of the crater shows ~2 µm diameter at the top.

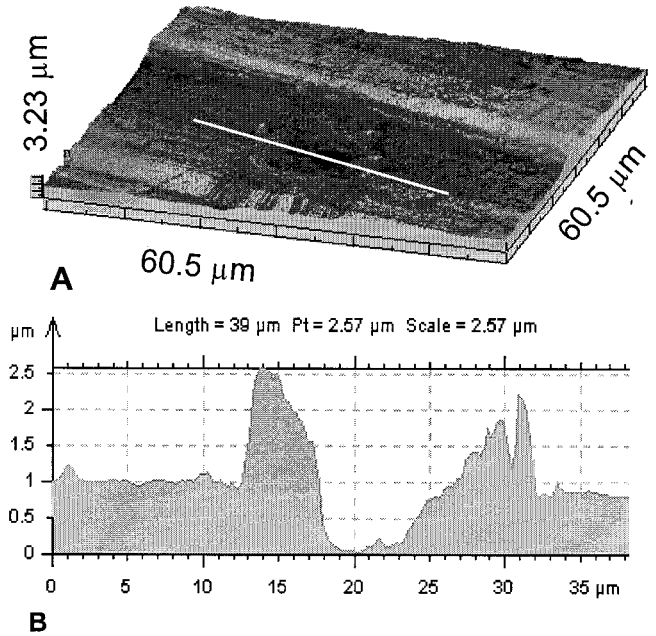

Figure 8. Topography (a) and cross section (b) of a large crater formed by a single laser pulse delivered through fiber probe to the surface of polished aluminum. Large crater rim was formed by melted metal.

4. Discussion

As was stated in the beginning of this paper, the goal of our study is to produce an instrument which would be useful for planetary exploration and life detection in particular. Keeping this in mind, we need not only to characterize the device by doing experiments with a host of samples, but also to put these studies in context of current global strategy for astrobiological exploration.

Once we have the instrument operational, the plan is to perform a series of head-to-head comparison experiments on a same set of samples with other instruments considered for life detection. We believe that complex astrobiological tasks cannot be addressed by a single instrument but rather by a suite of complementary tools. A broad comparison of different tools on a same sample set will allow defining the best instrument combination for tackling the life detection problem.

5. Conclusions

We are in the process of development and characterization of a new tool for spatially resolved chemical analysis. Ultimately, the Chemical Imager will be able to provide topographical and chemical maps of samples with sub-micron spatial resolution.

6. Acknowledgment

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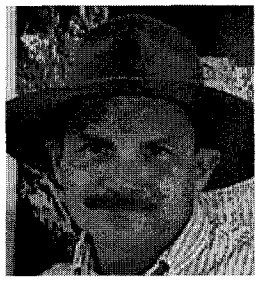
Dmitri Kossakovski *is a Member of Engineering Staff in the In-situ Experimental Science Group in Imaging and Spectrometry Section at NASA's Jet Propulsion Laboratory. He received his Ph.D. in Physical Chemistry from Caltech in 1999. He has pioneered several methods for chemical imaging with high spatial resolution based on a combination of Scanning Probe Microscopy with chemically sensitive techniques. Currently, Dmitri is working on a prototype Chemical Imager for astrobiological tasks for topographical and elemental sample mapping with sub-micron spatial resolution. He is also involved in projects on autonomous sample handling, real-time aerosol analysis and biological microarray analysis. He has authored/co-authored 9 peer-reviewed scientific papers and holds two U.S. patents.*

Gregory Bearman *is supervisor of the In-Situ Experimental Science Group in the Imaging and Spectrometry Section at NASA's Jet Propulsion Laboratory. His interests and work has recently been a combination of photonics technologies applied to biomedical problems and in-situ instrument technologies. Recent biomedical research includes work on retinal imaging spectroscopy in collaboration with Cedars-Sinai and corneal fluorescence with the University of California-Irvine and Cedars-Sinai. Dr. Bearman is a visiting scientist at Cedars-Sinai Medical Center, St.Lukes Hosptial in Houston and sits on the editorial board of the Journal of Biomedical Optics. He is conference chair for the SPIE BIOS topical meeting on biological spectroscopy.*



Joseph Kirschvink *is a Professor of Geobiology at California Institute of Technology. He is a Magnetogeobiologist, with research interests that include geomagnetic reversals, paleomagnetism, and magnetostratigraphy, geomagnetic sensitivity in animals, the biological effects of electromagnetic fields, biomineralization of ferrimagnetic materials. He is in charge of the Caltech Paleomagnetism and Biomagnetism Laboratories.*